METHODS FOR DIFFERENTIATING AND MONITORING PARATHYROID AND BONE STATUS RELATED DISEASES

TECHNICAL FIELD

The present invention relates to novel methods and devices for differentiating in a patient parathyroid diseases, such as hyperparathyroidism, from normal or non-disease states. One detects whole or non-fragmented (1 to 84) parathyroid hormone in a biological sample and also a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist: By either comparing values or using independently the value of either the large non-whole parathyroid hormone peptide fragment, the whole parathyroid hormone, or the combination of these values one can differentiate parathyroid and bone related disease states, as well as differentiate such states from normal states.

RELATED APPLICATIONS

The present application is a continuation-in-part of a non-provisional utility patent application filed in the United States Patent and Trademark Office, Serial Number 09/231,422.

BACKGROUND ART

Calcium plays an indispensable role in cell permeability, the formation of bones and teeth, blood coagulation, transmission of nerve impulse, and normal muscle contraction. The concentration of calcium ions in the blood is, along with calcitrol and calcitonin, regulated mainly by parathyroid hormone (PTH). Although calcium intake and excretion may vary, PTH serves through a feedback mechanism to maintain a steady concentration

of calcium in cells and surrounding fluids. When serum calcium lowers, the parathyroid glands secrete PTH, affecting the release of stored calcium. When serum calcium increases, stored calcium release is retarded through lowered secretions of PTH.

The complete form of human PTH, sometimes referred to in the art as hPTH but referred to in the present invention either as whole PTH or wPTH, is a unique 84 amino acid peptide (SEQ ID NO.1), as is shown in FIGURE 1. Researchers have found that this peptide has an anabolic effect on bone that involves a domain for protein kinase C activation (amino acid residues 28 to 34) as well as a domain for adenylate cyclase activation (amino acid residues 1 to 7). However, various catabolic forms of clipped or fragmented PTH peptides also are found in circulation, most likely formed by intraglandular or peripheral metabolism. For example, whole PTH can be cleaved between amino acids 34 and 35 to produce a (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment. Likewise, clipping can occur between either amino acids 36 and 37 or 37 and 38. Recently, a large PTH fragment referred to as "non-(1-84) PTH" has been disclosed which is clipped closer to the N-terminal end of PTH. (See R. LePage *et alia, "A non-(J-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples* " Clin. Chem. (1998); 44: 805-810.)

The clinical need for accurate measurement of PTH is well demonstrated. Serum PTH level is one of the most important indices for patients with the following diseases: familial hypocalciuria; hypercalcemia; multiple endocrine neoplasia types I and II; osteoporosis; Paget's bone disease; primary hyperparathyroidism -caused by primary hyperplasia or adenoma of the parathyroid glands; pseudohypoparathyroidism; and renal failure, which can cause secondary hyperparathyroidism.

PTH plays a role in the course of disease in a patient with chronic renal failure. Renal osteodystrophy (RO) is a complex skeletal disease comprising osteitis fibrosa cystica (caused by PTH excess), osteomalacia -unmineralized bone matrix (caused by

vitamin D deficiency), extraskeletal calcification/ossification (caused by abnormal calcium and phosphorus metabolism), and adynamic bone disease (contributed to by PTH suppression). Chronic renal failure patients can develop RO. Failing kidneys increase serum phosphorus (hyperphosphoremia) and decrease 1,25-dihydroxyvitamin D (1,25-D) production by the kidney. The former results in secondary hyperparathyroidism from decreased gastrointestinal calcium absorption and osteitis fibrosa cystica from increased PTH in response to an increase in serum phosphorus. The later causes hypocalcemia and osteomalacia. With the onset of secondary hyperparathyroidism, the parathyroid gland becomes less responsive to its hormonal regulators because of decreased expression of its calcium and vitamin D receptors. Serum calcium drops. RO can lead to digital gangrene, bone pain, bone fractures, and muscle weakness.

Determining circulating biologically active PTH levels in humans has been challenging. One major problem is that PTH is found at low levels, normally 10pg/mL to 65 pg/mL. Coupled with extremely low circulating levels is the problem of the heterogeneity of PTH and its many circulating fragments. In many cases, immunoassays have faced substantial and significant interference from circulating PTH fragments. For example, some commercially available PTH kits have almost 100% cross-reactivity with the non-(1-84) PTH fragment, (see the LePage article).

PTH immunoassays have varied over the years. One early approach is a double antibody precipitation immunoassay found in U. S. 4,369,138 to Arnold W. Lindall *et alia.* A first antibody has a high affinity for a (65-84) PTH fragment. A radioactive labeled (65-84) PTH peptide is added to the sample with the first antibody to compete for the endogenous unlabeled peptide. A second antibody is added which binds to any first antibody and radioactive labeled PTH fragment complex, thereby forming a precipitate. Both precipitate and supernatant can be measured for radioactive activity, and endogenous PTH levels can be calculated therefrom.

In an effort to overcome PTH fragment interference, immunoradiometric two-site assays for intact PTH (I-PTH) have been introduced, such as Allegro® Intact PTH assay by the Nichol's Institute of San Juan Capistrano, California. In one version, a capture antibody specifically binds to the C-terminal portion of hPTH while a labeled antibody specifically binds to the N-terminal portion of the captured hPTH. In another, two monoclonal antibodies were used, both of which attached to the N-terminal portion of hPTH. Unfortunately, these assays have problems in that they measure but do not discriminate between wPTH and non-whole PTH peptide fragments. This inability comes to the fore in hyperparathyroid patients and renal failure patients who have significant endogenous concentrations of large, non-whole PTH fragments.

Recently, researchers have made a specific binding assay directed to the large N-terminal PTH fragments. (See Gao, Ping *et alia "Immunochemicalluminometric assay with two monoc/ona antibodies against the N-termina/ sequence of human parathyroid hormone",* Clinica Chimica Acta 245 (1996) 39-59.) This immunochemiluminometric assay uses two monoclonal antibodies to detect N-terminal (1-34) PTH fragments but not mid-portion PTH fragments or C-terminal PTH fragments. A key factor in the design of these assays is to eliminate any reaction with C-terminal PTH fragments.

DISCLOSURE OF THE INVENTION

The present invention relates to novel methods and devices for differentiating in a patient parathyroid diseases, (such as primary hyperparathyroidism, secondary hyperparathyroidism, and stages thereof), from normal or non-disease states; for monitoring the function of parathyroid glands either during or after treatment, i.e., intra-operation and after operation parathyroid function monitoring as well as therapeutic treatment; and also for monitoring the effects of therapeutic treatments for parathyroid

related bone diseases and hyperparathyroidism. One detects the level in the serum or blood of at least one of three different parameters, namely, whole or non- fragmented parathyroid hormone in a biological sample, a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist, or the combination of the two values. By comparing the two values or by examining independently one of the above three values, one can differentiate parathyroid and bone disease states, as well as differentiate such states from normal states, as the relationship between these values, as well as the values themselves, change significantly between a normal person and a patient with a parathyroid disease.

The present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No.2 [PTH_{3-84}]) and (SEQ ID No.3 [PTH_{34-84}]), functions *in vivo* as a wPTH antagonist or inhibitor (PIN), (see FIGURE 12). In other words, the binding of wPTH to PTH receptors and the subsequent biological activity are affected by the presence of this PIN peptide fragment. The PTH receptors can be tied up with respect to PTH or PTH analogs in that the PTH binding site is blocked. The relationship between the concentrations of wPTH and PIN vary with PTH related disease states, and thus, are indicative of such states. Equally useful in view of the discovery of the antagonist nature of PIN, the present invention relates to novel methods and devices for monitoring parathyroid related bone diseases, and resultant bone loss or build-up. Increased amounts of PIN can inhibit the calcium releasing activity of PTH.

In making a measurement of wPTH, one does not want to detect PIN. The method for measuring the amount of wPTH in a sample such as serum, plasma, or blood comprises four general steps which can vary depending upon whether one uses a first antibody or antibody fragment specific for the PTH peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID No.4), wherein at least four amino acids are part of the antibody reactive portion of the peptide either as a signal antibody or a capture antibody in

conventional immunoassay formats. (One can also use an analogous peptide present in other species, such as a rat peptide in which the first amino acid serine is substituted with an alanine.) Used either as a signal antibody or as a capture antibody, enough antibody is added to bind all wPTH present. Next, one allows the first antibody to bind to any wPTH present, thereby forming a complex. A specific binding label comprised of a second antibody and a conventional immunoassay label, such as chemiluminescent agents, colorimetric agents, energy transfer agents, enzymes, fluorescent agents, and radioisotopes, is used to label the complex, preferably at the C-terminal end of wPTH, and can be added either substantially simultaneously with the first antibody or subsequent thereto. Finally, one uses conventional techniques to measure the amount of labeled complex, and thereby calculate wPTH levels in the sample. If used as a signal antibody, then the first antibody still attaches at the N-terminal end, but the second antibody would serve as a capture antibody that attaches at the C-terminal end.

In making a measurement of PIN, one can either measure it directly, or indirectly. An indirect measurement can be made by first measuring wPTH and then measuring total PTH. Subtracting the wPTH value from the total PTH value, one derives the PIN value. (For the purposes of the present invention, "total PTH" refers to the sum of wPTH, the naturally occurring predominant PTH receptor binding agonist, and PIN, the naturally occurring predominant PTH receptor binding antagonist.) A total PTH assay detects both PIN and wPTH by detecting the N-terminal end of PTH not at SEQ ID No.4, the very end of the N-terminal. By detecting between about amino acids 7 to 38 of PTH, the assay can detect both. A commercially available assay for total PTH is available from Scantibodies Laboratory, Inc. of Santee, California. A direct measurement of total PTH can be made by using an antibody or antibody fragment specific for a portion of the PTH peptide LEU-MET-HIS-ASN-LEU-GLY-LYS-HIS-LEU-ALA-SER-VAL-GLU-ARG-MET-GLN- TRP-LEU-ARG-LYS-LYS-LEU-GLN-ASP-VAL-HIS-ASN-PHE-VAL ALA-LEU-GLY (SEQ ID No.5), which comprises amino acids 7 to 38 of PTH, (preferably between amino acids 9 to 34), wherein at least four amino acids are part of the

antibody reactive portion of the peptide. Such an antibody or antibody fragment can be used in conventional immunoassay formats either as a signal antibody or a capture antibody.

To differentiate between parathyroid disease states and the normal state or to monitor the effects of therapeutic treatment for parathyroid disease states, one can compare the relationship between the values of wPTH, PIN, or total PTH, (the combination of wPTH and PIN), in other words, the relationship between the values of PIN and total PTH, between PIN and whole PTH, or between whole PTH and total PTH. For example, one can use a proportion between wPTH and total PTH, between PIN and total PTH, or between PIN and wPTH. (Comparisons can even take the form of a neural network of all these factors.) Regardless of the comparative method chosen, these values change significantly between a normal person and a patient with a parathyroid disease and between various stages of parathyroid diseases.

Alternatively, one can either differentiate between parathyroid disease states and the normal state or monitor the effects of therapeutic treatment for parathyroid disease states by examining independently the value of either wPTH, PIN, or total PTH alone.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a diagrammatic view of human wPTH.

FIGURE 2 is a diagrammatic view of a wPTH assay using the present antibody as a tracer element.

FIGURE 3 is a diagrammatic view of a wPTH assay using the present antibody as a capture element.

FIGURE 4 is a graph showing a standard curve for a wPTH assay.

FIGURE 5 is a graph comparing a conventional I-PTH assay with the present wPTH assay for healthy normal persons with "normal" PTH values.

FIGURE 6 is a diagrammatic view showing interference from PIN in conventional I-PTH assays.

FIGURE 7 is a graph comparing a conventional I-PTH assay with the present wPTH assay for patients with chronic uremia.

FIGURE 8 is a graph showing the distribution of wPTH values for healthy normal persons, patients with primary hyperparathyroidism, and patients with chronic uremia.

FIGURE 9 is a diagrammatic view showing how PIN blocks the action of wPTH at the receptor level, thereby making the person insensitive to the biological effects of wPTH.

FIGURE 10 is a graph demonstrating complete cross-reactivity of wPTH and PIN in a total PTH assay used in the present invention.

FIGURE 11 is a graph demonstrating how the whole PTH assay used in the present invention does not detect to PIN.

FIGURE 12 is a graph demonstrating how PIN is an *in vivo* inhibitor of wPTH.

BEST MODES FOR CARRYING OUT THE INVENTION

In disclosing the present invention, one should remember that there are a number of closely analogous, species dependent forms of PTH. The amino acid sequence of hPTH is shown in FIGURE 1. However, for rat PTH, bovine PTH, or porcine PTH, for example, one finds the substitutions at some of the amino acids in the hPTH sequence. For the purposes of the present invention, one can use interchangeably antibodies or antibody fragments to forms of these PTHs, although it is preferred to use an antibody with specificity for PTH having a sequence matching the species in which the PTH measurements are made.

Whole PTH immunoassay

A preferred embodiment of the present invention is an immunoradiometric assay (IRMA), often referred to as a sandwich assay, as shown FIGURES 2 and 3. Elements employed in such an assay (10) include a capture antibody (12) attached to a solid support (14) and a signal antibody (16) having a label (18), attached thereto (20). Typically, one selects a capture antibody that is specific for C-terminal PTH fragments (22), while the label antibody is specific for the initial wPTH peptide sequence which comprises a domain for adenylate cyclase activation (24), as shown in FIGURE 2. However, one could reverse the specificity of these antibodies, as is shown in FIGURE 3.

Alternatively, one could create an immunoassay in which wPTH is either precipitated from solution or otherwise differentiated in a solution, as in conventional precipitating assays or turbidometric assays. For example, one can use at least three antibodies to form a precipitating mass. In addition to the initial wPTH sequence antibody and a C-terminal antibody, one can use at least a third antibody which attaches to the mid portion of PTH. The combined mass of wPTH and the at least three antibodies would form a labeled precipitating mass which can be measured by conventional techniques.

Another method would be to couple the initial wPTH sequence antibody to colloidal solid supports, such as latex particles.

More specifically, one can create a signal antibody by iodinating 50 micrograms of affinity purified goat anti-(1-6) PTH antibody (Scantibodies Laboratory, Inc., Santee California, U.S.A.) by oxidation with chloramine T, incubation for 25 seconds at room temperature with 1 millicurie of 125-1 radioisotope and reduction with sodium metabisulfate. Unincorporated 125-1 radioisotope is separated from the 125-1-Goat anti-(1-6) PTH signal antibody by, passing the iodination mixture over a PD-l 0 desalting column (Pharmacia, Uppsala, Sweden) and following the manufacturers instructions. The fractions collected from the desalting column are measured in a gamma counter and those fractions representing the 125-1-goat anti-(1-6) PTH antibody are pooled and diluted to approximately 300,000 D PM (disintegrations per minute) per 100 microliters. This solution is the tracer solution to be used in the whole PTH IRMA.

Capture antibody coated tubes can be created by attaching affinity purified goat anti PTH 39-84 antibody, (Scantibodies Laboratory, Inc., Santee, California, U.S.A.), to 12 x 75 mm polystyrene tubes (Nunc, Denmark) by means of passive absorption techniques which are known to those of skill in the art. The tubes are emptied and dried, creating solid phase antibody coated tubes.

To conduct a whole PTH assay of a sample, 200 microliter samples of human serum are added to the solid phase antibody coated tubes. To each tube is added 100 microliters of the tracer solution (labeled goat anti-(1-6) PTH signal antibody). The tubes are incubated at room temperature with shaking at 170 rpm for 20-22 hours. During this time the immunochemical reaction of forming the sandwich of {solid phase goat anti-(39-84) PTH antibody} --{ whole PTH} --{ 125-1-goat anti-(1-6) PTH antibody} takes place. Following this incubation, the test tubes are washed with distilled water. Radioactivity on the solid phase, which amount corresponds to the quantity of wPTH present, is measured

using a gamma counter. The radioactivity data for the samples is processed by conventional analysis with use of the results from standards and controls and computer software in order that the concentration of whole PTH in the samples may be ascertained. FIGURE 4 shows a standard curve for such an assay.

Initial whole PTH sequence peptide

In order to make the signal antibody in the above assay, first one makes a synthetic PTH peptide corresponding either to hPTH (Ser -Val -Ser -Glu -Ile -Gln -Leu -Met), rat PTH (Ala -Val- Ser -Glu -Ile -Gln -Leu -Met), or at least four amino acids in the common sequence. The selected peptide can play two roles in making an assay, first as a specific source for creating a polyclonal antibody or monoclonal antibody source for signal antibody or capture antibody, and second as part of an affinity purification means for isolating the desired signal antibody or capture antibody.

Briefly, such a peptide can be synthesized on an Applied Biosystems, Inc. (Foster City, California, U.S.A.) Model 431 automated peptide synthesizer employing Fmoc (9-fluoronylmethoxycarbonyl) as the alpha-amino protecting group. All amino acids and solvents are from Applied Biosystems and are of synthesis grade. Following synthesis, the peptide is cleaved from the resin, and side chains are de-blocked, using a cleavage cocktail containing 6.67% phenol, 4.4% (v/v) thioanisole and 8.8% ethanedithiol in trifluoroacetic acid (TFA). The cleaved peptide is precipitated and washed several times in cold diethyl ether. It is then dissolved in water and lyophilized. The crude peptide is subjected to amino acid analysis (Waters PICO-TAG System, Boston, Massachusetts, U.S.A.) and reversed-phase HPLC using a VYDAC (TM) C8 column with 0.1% TF A in water and 99.9% acetonitrile in 0.1% TFA as the mobile buffers. The presence of a single major peak along with the appropriate amino acid composition is taken as evidence that the peptide is suitable for further use.

The resulting peptide is then attached to cross linked agarose beads (activated Sepharose 4B from Pharmacia, Uppsala, Sweden) according to instructions from the manufacturer. Armed with the initial peptide sequence on a bead, one can affinity purify a polyclonal antibody serum source to isolate the initial sequence antibody for the wPTH immunoassay.

Initial sequence whole PTH antibody

To create an affinity-purified anti-(1-6) PTH antibody, one first uses a selected initial PTH sequence peptide as described above as part of an immunogen for injection into a goat. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5,000 and 10,000,000, or as part of the wPTH complete sequence. The immunogen is mixed with an equal volume of Freunds complete adjuvant which is a mixture of light mineral oil, Arlacel detergent, and inactivated mycobacterium tuberculosis bacilli. The resulting mixture is homogenized to produce an aqueous/oil emulsion which is injected into the animal (typically a goat) for the primary immunization. The immunogen dose is approximately 50-400 micrograms. The goats are injected monthly with the same dose of immunogen complex except no mycobacterium tuberculosis bacilli is used in these subsequent injections. The goats are bled monthly, approximately three months after the primary immunization. The serum (or antiserum) is derived from each bleeding by separating the red blood cells from the blood by centrifugation and removing the antiserum which is rich in (1-6) PTH antibodies.

To purify the antiserum for the desired (1-6) PTH antibody, one packs a separation column with the initial PTH sequence peptide bound beads described above, washes the column and equilibrates it with 0.01 M phosphate buffered saline (PBS). The antiserum is loaded onto the column and washed with 0.01 M PBS in order to remove antibodies without the (1-6) PTH specificity. The bound specific goat anti-(1-6) PTH polyclonal antibody is eluted from the solid phase PTH 1-6 in the column by passing an elution

solution of 0.1 M glycine hydrochloride buffer, pH 2.5 through the column. The eluted polyclonal antibody is neutralized after it leaves the column with either the addition of 1.0 M phosphate buffer, pH 7.5 or by a buffer exchange with 0. 01 M PBS, as is known to those of skill in the art. The polyclonal antibody is stored at 2-8 degrees centigrade.

Comparison between whole PTH and total PTH assays

The present wPTH IRMA assay was compared to a conventional intact PTH or I-PTH immunoassay, the Allegro Nichols Intact-PTH assay, (which is commercially available and made by Nichols Institute Diagnostics of San Juan Capistrano, California, U.S.A.), in both PTH normal persons and those suffering from chronic uremia. This I-PTH immunoassay, due to its 100% cross reactivity between PIN and wPTH, is in actuality a total PTH assay, (see FIGURE 10).

FIGURE 5 shows the results for 34 normal human serum samples from healthy subjects which were assayed both by the present wPTH IRMA and the above I-PTH assay. In every case, the level of wpm detected by the IRMA is lower than that reported by the I-PTH assay, demonstrating the ability of the present IRMA to avoid detecting the interfering large, non (1-84) PTH fragment detected by the I-PTH assay, (see FIGURE 11). FIGURE 6 illustrates how such interference can occur. An N-terminal PTH specific signal antibody which is not specific to the initial PTH peptide sequence, as in the present invention, can detect not only wPTH (as in the upper part of FIGURE 6), but also can detect PIN, the large, non (1-84) PTH fragment, (as in the lower part of FIGURE 6).

A comparison of assay results for 157 chronic uremic patients is shown in FIGURE 7. Serum samples from these patients were measured using the wPTH IRMA and the above I-PTH assay. In every case the wPTH levels are lower than I-PTH values.

Clinical Use

The present wPTH and PIN assays have been used in a clinical setting involving 188 persons. The group included 31 persons having normal healthy parathyroid glands and 157 patients with chronic uremia who are undergoing dialysis on a continuous basis. Each person had a blood sample drawn which was assayed using a wPTH assay from Scantibodies Laboratory, Inc. as well as an I-PTH assay from Nichols Institute which gave total PTH values.

Table I shows the results individually and comparatively, of the wPTH, PIN, and total PTH assays from chronic uremic patients on dialysis.

TABLE 1

Patient No.	*Total PTH pg/ml*	*Whole PTH pg/ml*	*PIN pg/ml*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
1	1410	740	670	48%	91%	52%
2	185	89	96	52%	108%	48%
3	231	104	127	55%	122%	45%
4	1020	590	430	42%	73%	53%
5	270	159	111	41%	70%	59%
6	201	100	101	50%	101%	50%
7	380	100	280	74%	280%	26%
8	460	277	183	40%	66%	60%
9	380	197	183	48%	93%	52%
10	880	522	358	41%	69%	59%
11	310	154	156	50%	101%	50%
12	880	451	429	49%	95%	51%
13	670	418	252	38%	60%	63%
14	390	221	169	43%	76%	57%
15	170	108	62	36%	57%	64%
16	510	381	129	25%	34%	75%
17	200	67	133	67%	199%	34%
18	170	109	61	36%	56%	64%
19	360	199	161	45%	81%	55%
20	260	164	96	37%	59%	63%
21	440	372	68	15%	18%	85%

Patient No.	Total PTH pg/ml	Whole PTH pg/ml	PIN pg/ml	PIN to Total PTH	PIN to Whole PTH	Whole PTH to Total PTH
22	120	51.7	68.3	57%	132%	43%
23	600	527	73	12%	14%	83%
24	220	130	90	41%	69%	59%
25	190	136	54	28%	40%	72%
26	220	118	102	46%	86%	54%
27	630	334	296	47%	89%	53%
28	150	90	60	40%	67%	60%
29	170	106	64	38%	60%	62%
30	810	489	321	40%	66%	60%
31	570	319	251	44%	79%	56%
32	570	467	103	18%	22%	82%
33	400	300	100	25%	33%	75%
34	560	378	182	33%	48%	68%
35	310	121	189	61%	156%	39%
36	240	98	142	59%	145%	41%
37	280	133	157	54%	118%	48%
38	230	124	106	46%	85%	54%
39	350	319	31	9%	10%	91%
40	200	133	67	34%	50%	67%
41	920	564	356	39%	63%	61%
42	210	89	121	58%	136%	42%
43	1990	904	1086	55%	120%	45%
44	300	212	88	29%	42%	71%
45	260	132	128	49%	97%	51%
46	140	72	68	49%	94%	51%
47	250	129	121	48%	94%	52%
48	130	72	58	45%	81%	56%
49	1840	1000	840	46%	84%	54%
50	280	167	113	40%	68%	60%
51	490	268	222	45%	83%	55%
52	150	77.1	72.9	49%	95%	51%
53	140	58.1	81.9	59%	141%	42%
54	210	92.7	117.3	56%	127%	44%
55	160	79	81	51%	103%	49%
56	480	296	184	38%	62%	62%
57	480	281	199	41%	71%	59%
58	270	120	150	56%	125%	44%

Patient No.	*Total PTH pg/ml*	*Whole PTH pg/ml*	*PIN pg/ml*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
59	97	45	52	54%	116%	46%
60	330	154	176	53%	114%	47%
61	110	56	54	49%	96%	51%
62	660	456	204	31%	45%	69%
63	300	137	163	54%	119%	46%
64	240	145	95	40%	66%	60%
65	100	66.5	33.5	34%	50%	67%
66	410	416.3	-6.3	-2%	-2%	102%
67	410	235.7	174.3	43%	74%	57%
68	45	14.4	30.6	68%	213%	32%
69	200	102.3	97.7	49%	96%	51%
70	300	134	166	55%	124%	45%
71	320	202	118	37%	58%	63%
72	440	254	186	42%	73%	58%
73	190	99.6	90.4	48%	91%	52%
74	160	74.6	85.4	53%	114%	47%
75	600	429.8	170.2	28%	40%	72%
76	1140	632	508	45%	80%	55%
77	440	211	229	52%	109%	48%
78	450	276	174	39%	63%	61%
79	510	344	166	33%	48%	67%
80	190	62.8	127.2	67%	203%	33%
81	170	86	84	49%	98%	51%
82	180	103.4	76.6	43%	74%	57%
83	78	22.7	55.3	71%	244%	29%
84	230	117	113	49%	97%	51%
85	160	96	64	40%	67%	60%
86	220	89	131	60%	147%	40%
87	470	321.5	148.5	32%	46%	68%
88	310	137	173	56%	126%	44%
89	2050	1127	923	45%	82%	55%
90	930	414	516	55%	125%	45%
91	180	65	115	64%	177%	36%
92	560	238	322	58%	135%	43%
93	640	597	43	7%	7%	93%
94	590	382	208	35%	54%	65%
95	270	103	167	62%	162%	38%

Patient No.	*Total PTH pg/ml*	*Whole PTH pg/ml*	*PIN pg/ml*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
96	560	349	211	38%	60%	62%
97	180	78	102	57%	131%	43%
98	790	429	361	46%	84%	54%
99	670	372	298	44%	80%	56%
100	140	20.4	119.6	85%	586%	15%
101	190	117	73	38%	62%	62%
102	190	108	82	43%	76%	57%
103	430	217	213	50%	98%	50%
104	560	439	121	22%	28%	78%
105	500	357.7	142.3	28%	40%	72%
106	1560	777	783	50%	101%	50%
107	62	24.3	37.7	61%	155%	39%
108	430	226	204	47%	90%	53%
109	160	67.2	92.8	58%	138%	42%
110	530	346	184	35%	53%	65%
111	260	142	118	45%	83%	55%
112	580	163	417	72%	256%	28%
113	440	579	-139	-32%	-24%	132%
114	500	232.3	267.7	54%	115%	46%
115	160	60	100	63%	167%	38%
116	340	202	138	41%	68%	59%
117	260	138	122	47%	88%	53%
118	260	119	141	54%	118%	46%
119	160	84	76	48%	90%	53%
120	130	46	84	65%	183%	35%
121	190	104	86	45%	83%	55%
122	420	334	86	20%	26%	80%
123	630	440	190	30%	43%	70%
124	75	26.4	48.6	65%	184%	35%
125	260	143	117	45%	82%	55%
126	640	409	231	36%	56%	64%
127	130	66.7	63.3	49%	95%	51%
128	700	381	319	46%	84%	54%
129	560	376	184	33%	49%	67%
130	240	107	133	55%	124%	45%
131	110	63	47	43%	75%	57%
132	420	297	123	29%	41%	71%

Patient No.	*Total PTH pg/ml*	*Whole PTH pg/ml*	*PIN pg/ml*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
133	580	229	351	61%	153%	39%
134	310	201.2	108.8	35%	54%	65%
135	160	97.9	62.1	39%	63%	61%
136	290	138.7	151.3	52%	109%	48%
137	200	96.2	103.8	52%	108%	48%
138	770	662.7	107.3	14%	16%	86%
139	290	130.7	159.3	55%	122%	45%
140	260	219	41	16%	19%	84%
141	350	211	139	40%	66%	60%
142	730	463.5	266.5	37%	57%	63%
143	490	231	259	53%	112%	47%
144	160	87	73	46%	84%	54%
145	380	222	158	42%	71%	58%
146	210	93.5	116.5	55%	125%	45%
147	630	383.4	246.6	39%	64%	61%
148	150	83.2	66.8	45%	80%	55%
149	320	152.5	167.5	52%	110%	48%
150	900	467.6	432.4	48%	92%	52%
151	1180	818.6	361.4	31%	44%	69%
152	120	38.4	81.6	68%	213%	32%
153	5230	1388	3842	73%	277%	27%
154	34	10.5	23.5	69%	224%	31%
155	1020	590.6	429.4	42%	73%	58%
156	280	76.6	103.4	57%	135%	43%
157	120	51.1	68.9	57%	135%	43%
Median	300	154	127	46%	84%	54%

TABLE 2 shows the results, individually and comparatively, of the wPTH, PIN, and total PTH assays from the normals.

TABLE 2

Patient No.	*Total PTH pg/ml*	*Whole PTH pg/ml*	*PIN pg/ml*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
1	17.13	3.32	13.81	81%	416%	19%
2	32.92	10.49	22.43	68%	214%	32%
3	31.32	10.31	21.01	67%	204%	33%
4	41.84	12.72	29.12	70%	229%	30%
5	33.03	10.09	22.94	69%	227%	31%
6	44.32	14.23	30.09	68%	211%	32%
7	31.47	6.80	24.67	78%	363%	22%
8	20.82	10.03	10.79	52%	108%	48%
9	34.64	15.95	18.69	54%	117%	46%
10	23.69	5.25	18.44	78%	351%	22%
11	53.98	17.82	36.16	67%	203%	33%
12	52.71	18.83	33.88	64%	180%	36%
13	26.92	5.63	21.29	79%	378%	21%
14	39.93	11.86	28.07	70%	237%	30%
15	48.84	20.47	28.37	58%	139%	42%
16	29.56	13.68	15.88	54%	116%	46%
17	36.19	14.69	21.50	59%	146%	41%
18	20.96	6.99	13.97	67%	200%	33%
19	59.29	27.89	31.40	53%	113%	47%
20	45.57	18.23	27.34	60%	150%	40%
21	35.64	18.72	16.92	47%	90%	53%
22	38.53	19.56	18.97	49%	97%	51%
23	21.71	9.34	12.37	57%	132%	43%
24	32.42	13.51	18.91	58%	140%	42%
25	28.50	10.41	18.09	63%	174%	37%
26	18.17	7.80	10.37	57%	133%	43%
27	39.96	17.29	22.67	57%	131%	43%
28	34.08	15.24	18.84	55%	124%	45%
29	42.95	19.59	23.36	54%	119%	46%
30	38.40	12.16	26.24	68%	216%	32%
31	47.57	18.45	29.12	61%	158%	39%
MEDIAN	34.64	13.51	21.50	61%	158%	39%

Clearly, the statistically significant differences in the medians of these two groups demonstrates that one can differentiate between the two by using these assays alone or by comparing their respective values.

TABLE 3

Sample Type	*Total PTH (pg/mL)*	*Whole PTH (pg/mL)*	*PIN (pg/mL)*	*PIN to Total PTH*	*PIN to Whole PTH*	*Whole PTH to Total PTH*
Chronic uremia (n=157) Medians	300	154	127	46%	84%	55%
Normal (n=31) Medians	34.64	13.51	21.50	61%	158%	37%
P-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.